CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF

PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.13899430

https://www.iajps.com/volumes/volume11-october-2024/07-issue-10-october-24/

Available online at: http://www.iajps.com *Research Article*

# VALIDATED RP-HPLC METHOD DEVELOPMENT FOR THE ESTIMATION OF DECITABINE & CEDAZURIDINE IN ITS COMBINED DOSAGE FORMS.

**A. Mahesh*, Mrs. K. Radhika, R. Madhulika, Dr. K. Shravankumar**

[1]Department Of Pharmaceutical Analysis, Samskruti College Of Pharmacy, Ghatkesar, Telangana. 501301.

**Article Received: August 2024 Accepted: September 2024 Published: October 2024**

**Abstract:**

*A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Decitabine and Cedazuridine in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Water (45:55% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240nm. The retention time of the Decitabine and Cedazuridine was found to be was 2.133, 3.692 ± 0.02min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Decitabine and 10-30mg/ml of Cedazuridine. The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.*

**Keywords:** *Decitabine and Cedazuridine, RP-HPLC, Validation, Accuracy, Precision.*

**Corresponding author:**

**A.Mahesh,**

*Department of Pharmaceutical Analysis,*
*Samskruti college of pharmacy,*
*Ghatkesar, Telangana.*
*Email Id- maheshavirendla1@gmail.com*

QR code

*Please cite this article in press* **A.Mahesh***et al* ***Validated RP-HPLC Method Development For The Estimation Of Decitabine & Cedazuridine In Its Combined Dosage Forms..,****Indo Am. J. P. Sci, 2024; 11 (10).*

## INTRODUCTION:

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

**Method development:**

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use" [1-2]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these show unique selectivity. What is needed is a more informed decision making process for column selection that may be used before the chromatographer enters the laboratory. The method of column selection presented here involves a minimal investment in time initially, with the potential of saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [3, 4].

The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization [5]. The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [6-8].

The degraded drug samples obtained are subjected to preliminary chromatographic separation to study the number and types of degradation products formed under various conditions [9]. Scouting experiments are run and then conditions are chosen for further optimization [10]. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development [11]. Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds. Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time. However, by operating at pH extremes (2-4 or 8-10), not only is there a 10-30 fold difference in retention time that can be exploited in method development but also the method can be made more robust which is a desirable outcome with validation in minutes. Various steps for HPLC method development are given below.

**Requirements for good method development:**
**Choosing the appropriate HPLC column:**
C18 columns are the commonly used columns in HPLC method analysis. C8 or Octyl bonded phases are also used occasionally. Like C18, they are non-polar, but not as hydrophobic. Therefore, retention times for hydrophobic compounds are typically shorter. Also, they may show somewhat different selectivity than C18 due to increased base silica exposure unique selectivity results in proton interaction of the bonded phase with electron deficient functional groups of solute molecules.

Retention is a mixed mechanism, resulting from both hydrophobic interactions and dipole interactions of the bonded phase C□N group with solute amino groups or p - p interactions with sites of unsaturation. It is the best for polar organic compounds and is versatile enough for use in both normal and reversed phase modes.
Each bonded phase has unique selectivity for certain sample types. For example: to separate toluene and ethyl benzene (differ by only one -$CH_2$- unit), we would choose a C18 bonded phase. Further, we would want to narrow the decision to a particular packing material that shows good or excellent retention of such hydrophobic compounds (i.e. high % carbon load) to be able to maximize the particular separation. The effects of surface area and carbon load are discussed in the next section. The stationary phase must be able to "hold on" to the two compounds long enough to resolve them.

**Column Dimensions:**
This refers to the length and internal diameter of the packing media bed within the column tube. Short columns (30-50mm) offer short run times, fast equilibration, low back pressure and high sensitivity. Long columns (250-300mm) provide higher resolving power, but create more backpressure, lengthen analysis times and use more solvent. Narrow column (2.1mm and smaller) beds inhibit sample diffusion and produce narrower, taller peaks and a lower limit of detection. They may require instrument modification to minimize distortion of the chromatography. Wider columns (10-22mm) offer the ability to load more sample.

**Particle shape:**
Most modern chromatographic packings have spherical particles, but some are irregular in shape. Spherical particles offer reduced back pressures and longer column life when using viscous mobile phases like 50:50 MeOH: $H_2O$.

**Particle Size:**
This refers to the average diameter of the packing media particles. Standard particle sizes range from 3µm (high efficiency) to 15-20µm (preparative). A 5µm particle size offers a good compromise between efficiency and back pressure. Smaller particles pack into columns with a higher density, allowing less diffusion of sample bands between particles and causing narrower, sharper peaks. However, smaller particles also cause higher solvent back pressures. As a rule of thumb, 1.5 or 3µm particle sizes are to chosen for resolving complex, multi-component samples. Otherwise, 5 or 10µm packings should be considered.

**Surface area:**
Expressed in m2/gram, the total surface area of a particle is the sum of the outer particle surface and the interior pore surface. Solute retention is greater on packings that have a high surface area. High surface areas generally provide longer retention, greater capacity and higher resolution. As a rule of thumb, a base material with maximum surface area is to be used for resolving complex and multi-component samples.

**Pore size:**
This refers to the average size of the pores or cavities present in porous packing particles.Pore sizes range from 60Å on the low end to greater than 10,000Å on the high end. Larger pores allow larger solute molecules to be retained longer through maximum exposure to the surface area of the particles. A pore size of 150Å or less is chosen for sample MW □ 2000. For sample with molecular weight greater than 2000, columns with a pore size of 300Å or greater are to be used.

## MATERIALS AND METHODS:
Decitabine-Procured from Sun pharma, provided by Sura Pharma labs, Cedazuridine-Procured from Sun pharma, provided by Sura Pharma labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-

Merck, Potassium Dihydrogen Phosphate-Finar Chemicals.
**Hplc method development:**
**Trails**
**Preparation of standard solution:**
Accurately weigh and transfer 10 mg of Decitabine & Cedazuridine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and

sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Decitabine and 0.3ml of the Cedazuridine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Procedure:**
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

**Mobile Phase Optimization**:
Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 45:55 v/v respectively.

**Optimization of Column:**
The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

**Optimized chromatographic conditions**:
Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Mobile phase : Acetonitrile: Water (45:55 v/v)
Flow rate : 1ml/min
Wavelength : 240 nm
Injection volume : 10 µl
Run time : 6 min

**Validation**
**Preparation of buffer and mobile phase:**
**Preparation of mobile phase:**
Accurately measured 450 ml (45%) of Methanol, 550 ml of Water (55%) were mixed and degassed in digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
**Diluent Preparation:**
The Mobile phase was used as the diluent.

## RESULTS AND DISCUSSION:
**Optimized Chromatogram (Standard)**
Mobile phase ratio : Methanol: Water (45:55 v/v)
Column : Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Column temperature : 35ºC
Wavelength : 240nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

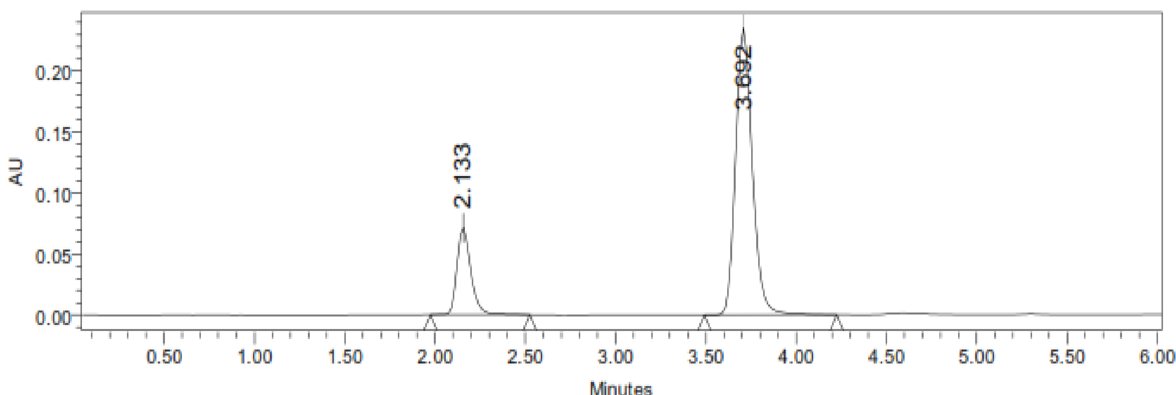


**Figure: Optimized Chromatogram (Standard)**

**Table: Optimized Chromatogram (Standard)**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.133 | 526388 | 86757 | 1.57 | 5678 | |
| 2 | Cedazuridine | 3.692 | 1687286 | 367533 | 1.78 | 8686 | 9.9 |

**Observation**: From the above chromatogram it was observed that the Cedazuridine and Decitabine peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So, it's optimized trial.

**Optimized Chromatogram**

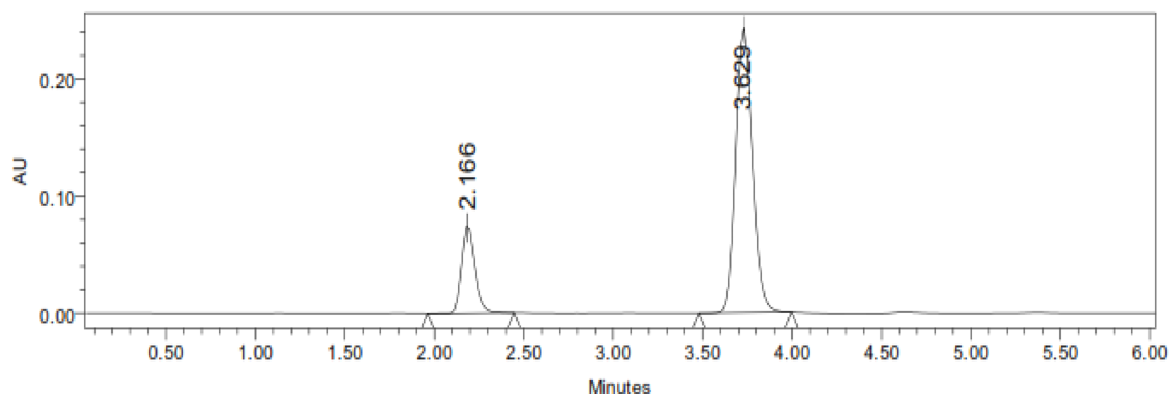


**Figure: Optimized Chromatogram (Sample)**

**Table: Optimized Chromatogram (Sample)**

| S.No. | Name | Rt | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.166 | 536588 | 77465 | 1.58 | 5788 | |
| 2 | Cedazuridine | 3.629 | 1695847 | 378563 | 1.81 | 8796 | 10.02 |

**Acceptance criteria:**

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

**System Suitability:**

**Table-: Results of system suitability for Decitabine**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.152 | 526359 | 86599 | 5696 | 1.58 |
| 2 | Decitabine | 2.157 | 526547 | 86255 | 5653 | 1.59 |
| 3 | Decitabine | 2.141 | 526853 | 86600 | 5628 | 1.58 |
| 4 | Decitabine | 2.133 | 526597 | 86246 | 5693 | 1.59 |
| 5 | Decitabine | 2.166 | 524878 | 86522 | 5642 | 1.58 |
| **Mean** | | | 526246.8 | | | |
| **Std. Dev.** | | | 785.2453 | | | |
| **% RSD** | | | 0.149216 | | | |

**Acceptance Criteria:**

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

**Table-: Results of system suitability for Cedazuridine**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Cedazuridine | 3.674 | 1682822 | 1686959 | 8658 | 1.56 | 9.8 |
| 2 | Cedazuridine | 3.631 | 1682727 | 1685746 | 8676 | 1.57 | 9.9 |
| 3 | Cedazuridine | 3.625 | 1687362 | 1685422 | 8693 | 1.56 | 9.8 |
| 4 | Cedazuridine | 3.692 | 1682812 | 1685243 | 8641 | 1.57 | 9.8 |
| 5 | Cedazuridine | 3.629 | 1683817 | 1685365 | 8636 | 1.58 | 9.8 |
| Mean | | | 1683908 | | | | |
| Std. Dev. | | | 1982.03 | | | | |
| % RSD | | | 0.117704 | | | | |

**Acceptance Criteria:**

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

**Assay (Standard):**

**Table: Peak results for assay standard of Decitabine**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.152 | 526357 | 86599 | 1.55 | 5699 | 1 |
| 2 | Decitabine | 2.198 | 526585 | 86783 | 1.58 | 5688 | 2 |
| 3 | Decitabine | 2.179 | 529659 | 86254 | 1.56 | 5638 | 3 |

**Table: Peak results for assay standard of Cedazuridine**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Cedazuridine | 3.646 | 1687588 | 365878 | 1.80 | 8658 | 1 |
| 2 | Cedazuridine | 3.604 | 1685986 | 365853 | 1.79 | 8696 | 2 |
| 3 | Cedazuridine | 3.610 | 1685973 | 369855 | 1.80 | 8674 | 3 |

**Assay (Sample):**

**Table: Peak results for Assay sample of Decitabine**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.152 | 536858 | 87583 | 1.58 | 5788 | 1 |
| 2 | Decitabine | 2.150 | 532653 | 87964 | 1.59 | 5783 | 2 |
| 3 | Decitabine | 2.187 | 532684 | 87466 | 1.58 | 5768 | 3 |

**Table: Peak results for Assay sample of Cedazuridine**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Cedazuridine | 3.646 | 1698569 | 378563 | 1.81 | 8758 | 1 |
| 2 | Cedazuridine | 3.651 | 1698573 | 375848 | 1.80 | 8796 | 2 |
| 3 | Cedazuridine | 3.601 | 1698548 | 376585 | 1.81 | 8744 | 3 |

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

The % purity of Cedazuridine & Decitabine in pharmaceutical dosage form was found to be 99.89%

**Linearity**

**Chromatographic data for linearity study:**

**Table-: Chromatographic Data for Linearity Study of Decitabine**

| Concentration μg/ml | Average Peak Area |
|---|---|
| 20 | 272898 |
| 30 | 402987 |
| 40 | 526388 |
| 50 | 649786 |
| 60 | 769288 |

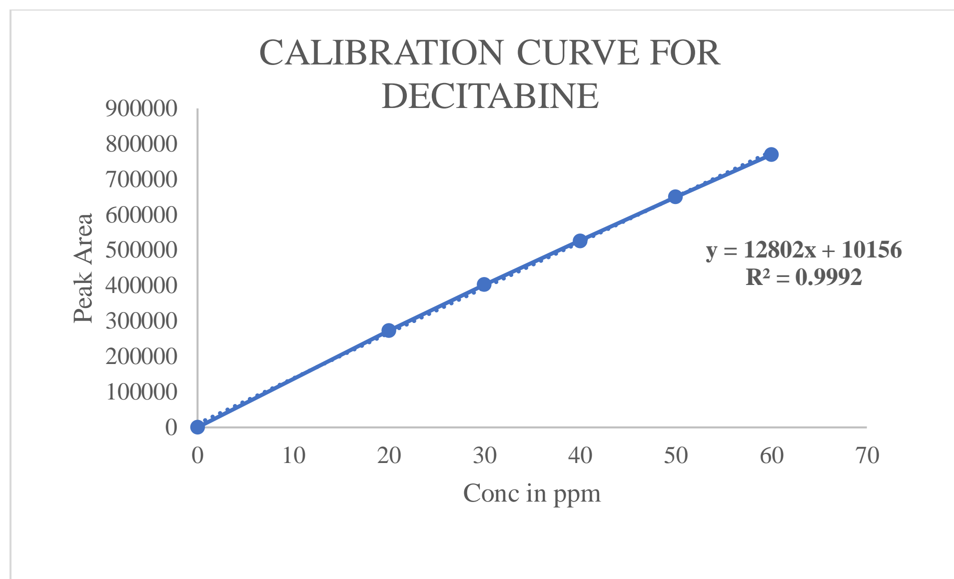


**Fig: Calibration Curve of Decitabine**

**Table-: Chromatographic Data for Linearity Study of Cedazuridine**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 10 | 1000238 |
| 15 | 1448769 |
| 20 | 1887286 |
| 25 | 2365898 |
| 30 | 2826846 |

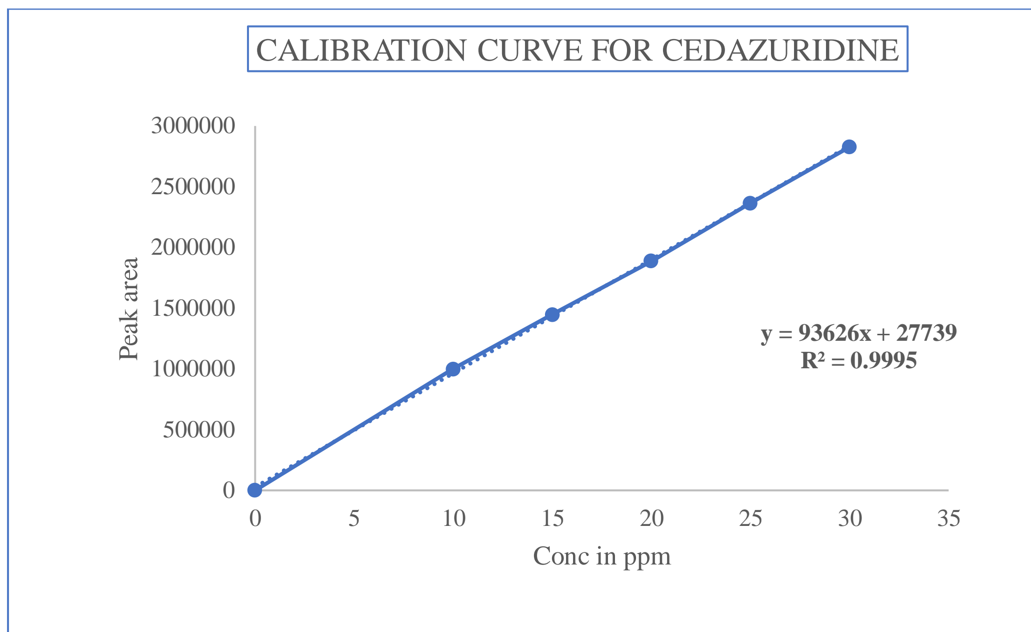


**Fig: Calibration Curve of Cedazuridine**

**Repeatability:**

**Table: Results of repeatability for Decitabine:**

| S. No. | Peak Name | Retention time | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.157 | 526359 | 86599 | 5688 | 1.56 |
| 2 | Decitabine | 2.159 | 524857 | 86543 | 5686 | 1.57 |
| 3 | Decitabine | 2.186 | 526986 | 86579 | 5685 | 1.56 |
| 4 | Decitabine | 2.160 | 528653 | 86355 | 5679 | 1.56 |
| 5 | Decitabine | 2.170 | 528456 | 86959 | 5638 | 1.56 |
| **Mean** | | | 527063 | | | |
| **Std.dev** | | | 1568.114 | | | |
| **%RSD** | | | 0.297708 | | | |

**Acceptance Criteria:**

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

**Table: Results of Repeatability for Cedazuridine:**

| S. No. | Peak Name | Retention time | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Cedazuridine | 3.603 | 1687588 | 367858 | 8658 | 1.79 |
| 2 | Cedazuridine | 3.608 | 1685986 | 368546 | 8677 | 1.80 |
| 3 | Cedazuridine | 3.600 | 1685988 | 367984 | 8646 | 1.80 |
| 4 | Cedazuridine | 3.696 | 1685753 | 365873 | 8694 | 1.79 |
| 5 | Cedazuridine | 3.629 | 1685986 | 364588 | 8627 | 1.79 |
| **Mean** | | | 1686260 | | | |
| **Std.Dev** | | | 749.493 | | | |
| **%RSD** | | | 0.044447 | | | |

**Intermediate precision:**

**Table: Results of Intermediate precision for Decitabine**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing | %Assay |
|---|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.198 | 546586 | 87588 | 5899 | 1.58 | 100% |
| 2 | Decitabine | 2.196 | 548757 | 87986 | 5878 | 1.59 | 100% |
| 3 | Decitabine | 2.160 | 549855 | 87453 | 5869 | 1.58 | 100% |
| 4 | Decitabine | 2.160 | 548799 | 87422 | 5848 | 1.59 | 100% |
| 5 | Decitabine | 2.160 | 542658 | 87964 | 5897 | 1.58 | 100% |
| 6 | Decitabine | 2.186 | 548755 | 87253 | 5875 | 1.59 | 100% |
| **Mean** | | | 547569 | | | | |
| **Std. Dev.** | | | 2632.576 | | | | |
| **% RSD** | | | 0.480594 | | | | |

**Acceptance criteria:**

- %RSD of five different sample solutions should not more than 2

**Table: Results of Intermediate precision for Cedazuridine**

| S.No. | Peak Name | Rt | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing | Resolution | %Assay |
|---|---|---|---|---|---|---|---|---|
| 1 | Cedazuridine | 3.623 | 1698588 | 385483 | 8788 | 1.81 | 9.8 | 98% |
| 2 | Cedazuridine | 3.611 | 1698575 | 385699 | 8757 | 1.80 | 9.8 | 98.1% |
| 3 | Cedazuridine | 3.696 | 1698533 | 385747 | 8755 | 1.81 | 9.9 | 98.8% |
| 4 | Cedazuridine | 3.696 | 1698575 | 386959 | 8756 | 1.81 | 10.01 | 99.8% |
| 5 | Cedazuridine | 3.696 | 1698533 | 385756 | 8799 | 1.80 | 9.98 | 98.6% |
| 6 | Cedazuridine | 3.642 | 1698548 | 386559 | 8763 | 1.80 | 10.02 | 98.3% |
| **Mean** | | | 1698559 | | | | | |
| **Std. Dev.** | | | 23.77114 | | | | | |
| **% RSD** | | | 0.001398 | | | | | |

**Acceptance criteria:**

- %RSD of five different sample solutions should not more than 2

**Table-: Results of Intermediate precision Day 2 for Decitabine**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Decitabine | 2.198 | 536855 | 8757 | 5788 | 1.58 |
| 2 | Decitabine | 2.196 | 536986 | 8796 | 5727 | 1.59 |
| 3 | Decitabine | 2.178 | 536588 | 8747 | 5743 | 1.58 |
| 4 | Decitabine | 2.142 | 532547 | 8753 | 5747 | 1.59 |
| 5 | Decitabine | 2.177 | 534588 | 8726 | 5799 | 1.58 |
| 6 | Decitabine | 2.177 | 538599 | 8727 | 5786 | 1.59 |
| **Mean** | | | 536027.2 | | | |
| **Std. Dev.** | | | 2132.492 | | | |
| **% RSD** | | | 0.397648 | | | |

**Acceptance criteria:**

- %RSD of five different sample solutions should not more than 2.

**Table-: Results of Intermediate precision Day 2 for Cedazuridine**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate | USP Tailing | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Cedazuridine | 3.611 | 1678599 | 356876 | 8876 | 1.82 | 9.9 |
| 2 | Cedazuridine | 3.623 | 1678986 | 358984 | 8857 | 1.83 | 10.01 |
| 3 | Cedazuridine | 3.684 | 1678983 | 358753 | 8863 | 1.82 | 9.9 |
| 4 | Cedazuridine | 3.697 | 1678986 | 352414 | 8848 | 1.83 | 10.01 |
| 5 | Cedazuridine | 3.684 | 1678548 | 358988 | 8874 | 1.82 | 9.9 |
| 6 | Cedazuridine | 3.684 | 1678985 | 358987 | 8843 | 1.83 | 10.01 |
| **Mean** | | | 1678849 | | | | |
| **Std. Dev.** | | | 213.8048 | | | | |
| **% RSD** | | | 0.012677 | | | | |

**Acceptance criteria:**

- %RSD of five different sample solutions should not more than 2.

**Accuracy:**

**Table: The accuracy results for Decitabine**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 267012.3 | 20 | 20.064 | 100.316% | 100.29% |
| 100% | 523753.3 | 40 | 40.119 | 100.296% | |
| 150% | 778458.3 | 60 | 60.134 | 100.222% | |

**Acceptance Criteria:**

- The percentage recovery was found to be within the limit (98-102%).

**Table: The accuracy results for Cedazuridine**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 972876.3 | 10 | 10.094 | 100.94% | 100.48% |
| 100% | 1900122 | 20 | 19.998 | 99.99% | |
| 150% | 2851152 | 30 | 30.156 | 100.52% | |

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

**Robustness**
**Decitabine:**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 526388 | 2.133 | 5678 | 1.56 |
| Less Flow rate of 0.9 mL/min | 542686 | 2.210 | 5263 | 1.54 |
| More Flow rate of 1.1 mL/min | 526484 | 2.184 | 5427 | 1.52 |
| Less organic phase | 516855 | 2.200 | 5164 | 1.57 |
| More Organic phase | 506899 | 2.172 | 5099 | 1.51 |

**Acceptance criteria:**
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

**Cedazuridine:**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 1687286 | 3.692 | 8686 | 1.78 |
| Less Flow rate of 0.9 mL/min | 1725469 | 4.498 | 8267 | 1.69 |
| More Flow rate of 1.1 mL/min | 1652848 | 3.505 | 8416 | 1.58 |
| Less organic phase | 1687486 | 4.504 | 8327 | 1.61 |
| More organic phase | 1674523 | 3.512 | 8416 | 1.63 |

**Acceptance criteria:**
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

## CONCLUSION:

In this study, a new RP-HPLC method was developed and validated for the simultaneous quantification of Decitabine and Cedazuridine in both their pure forms and combined marketed formulations. Chromatographic separation was achieved using a Phenomenex Luna C18 column with a mobile phase consisting of Methanol and Water (45:55% v/v), flowing at a rate of 1.0 ml/min, and detection was performed at 240 nm. The retention times observed for Decitabine and Cedazuridine were 2.133 and 3.692 ± 0.02 minutes, respectively.

The method was rigorously validated following ICH guidelines, covering key parameters such as linearity, sensitivity, accuracy, precision, specificity, and robustness. The calibration curves demonstrated linear responses over the concentration ranges of 20-60 mg/ml for Decitabine and 10-30 mg/ml for Cedazuridine. Both inter-day and intra-day precision studies indicated consistent results within acceptable limits, with method precision for assay determination yielding below 2.0% RSD.

Overall, this newly developed RP-HPLC method offers a straightforward, rapid, and precise approach for the quantitative analysis of Decitabine and Cedazuridine, making it suitable for routine quality control applications in bulk drug manufacturing and pharmaceutical formulations.

The validated RP-HPLC method presented in this study provides a reliable means for the simultaneous estimation of Decitabine and Cedazuridine in their individual and combined dosage forms. The method's robustness, demonstrated by its ability to separate and quantify both compounds efficiently and accurately, meets the stringent criteria set forth by ICH guidelines. The observed linear responses, excellent precision, and low method variability underscore its suitability for use in pharmaceutical quality control laboratories. By offering simplicity and rapid analysis without compromising precision, this method ensures dependable results essential for maintaining the quality and consistency of Decitabine and Cedazuridine formulations in the pharmaceutical industry.

**Acknowledgement:**
The Authors are thankful to the Management and Principal, Department of Pharmacy, Samskruti college of pharmacy in Ghatkesar, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

## BIBLIOGRAPHY:

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. D. H. Shewiy, E. Kaale, P. G. Risha, B. Dejaegher, J. S. Verbeke, Y. V. Heyden, Journal Pharmaceut. Biomed. Anal, 66, 2012, 11-23.
9. M. D. Rockville, General Tests, Chapter 621 – Chromatography System Suitability, United States Pharmacopeial Convention (USP), USP 31, 2009.
10. FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Centre for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER), 2000.
11. Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modelling in HPLC method development. J Adv Res, 3, 2012, 53-63.
12. Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. Lc Gc N. Am, 75, 2002, 49-50.
13. Snyder LR, Kirkland JJ, Glajach JL. X. In Practical HPLC Methods Development. John Wiley, New York, 295, 1997, 643-712.
14. Swartz M, Murphy MB. New Fronties in Chromatography. Am Lab, 37, 2005, 22-27.
15. Dolan JW. Peak tailing and resolution. Lc Gc N. Am, 20, 2002, 430-436.